Impact of bicarbonate, ammonium chloride, and acetazolamide on hepatic and renal SLC26A4 expression

Alesutan, I ; Daryadel, A ; Mohebbi, N ; Pelzl, L ; Leibrock, C ; Voelkl, J ; Bourgeois, S ; Dossena, S ; Nofziger, C ; Paulmichl, M ; Wagner, Carsten A ; Lang, F

Abstract: SLC26A4 encodes pendrin, a transporter exchanging anions such as chloride, bicarbonate, and iodide. Loss of function mutations of SLC26A4 cause Pendred syndrome characterized by hearing loss and enlarged vestibular aqueducts as well as variable hypothyroidism and goiter. In the kidney, pendrin is expressed in the distal nephron and accomplishes HCO(3)(-) secretion and Cl(-) reabsorption. Renal pendrin expression is regulated by acid-base balance. The liver contributes to acid-base regulation by producing or consuming glutamine, which is utilized by the kidney for generation and excretion of NH(4)(+), paralleled by HCO(3)(-) formation. Little is known about the regulation of pendrin in liver. The present study thus examined the expression of Slc26a4 in liver and kidney of mice drinking tap water without or with NaHCO(3) (150 mM), NH(4)Cl (280 mM) or acetazolamide (3.6 mM) for seven days. As compared to Gapdh transcript levels, Slc26a4 transcript levels were moderately lower in liver than in renal tissue. Slc26a4 transcript levels were not significantly affected by NaHCO(3) in liver, but significantly increased by NaHCO(3) in kidney. Pendrin protein expression was significantly enhanced in kidney and reduced in liver by NaHCO(3). Slc26a4 transcript levels were significantly increased by NH(4)Cl and acetazolamide in liver, and significantly decreased by NH(4)Cl and by acetazolamide in kidney. NH(4)Cl and acetazolamide reduced pendrin protein expression significantly in kidney, but did not significantly modify pendrin protein expression in liver. The observations point to expression of pendrin in the liver and to opposite effects of acidosis on pendrin transcription in liver and kidney.

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Impact of bicarbonate, ammonium chloride, and acetazolamide on hepatic and renal Slc26a4 expression

Ioana Alesutan[1*], Arezoo Daryadel[2*], Nilufar Mohebbi[2], Lisann Pelzl[1], Christina Leibrock[1], Jakob Voelkl[1], Soline Bourgeois[2], Silvia Dossena[3], Charity Nofziger[3], Markus Paulmichl[3], Carsten A. Wagner[2], Florian Lang[1]

*I. Alesutan and A. Daryadel contributed equally to this manuscript and therefore share first authorship

[1]Department of Physiology, University of Tuebingen, Germany. [2]Institute of Physiology and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland, [3]Institute of Pharmacology and Toxicology, Paracelsus Medical University, A-5020 Salzburg, Austria.

Short title: Regulation of Slc26a4

Address for correspondence:

Florian Lang

Department of Physiology

University of Tübingen

Gmelinstr. 5

72076 Tübingen

Germany

Phone: +49 7071 29-72194

Fax: +49 7071 29-5618

E-mail: florian.lang@uni-tuebingen.de

Abstract

SLC26A4 encodes pendrin, a transporter exchanging anions such as chloride, bicarbonate, and iodide. Loss of function mutations of SLC26A4 cause Pendred syndrome characterized by hearing loss and enlarged vestibular aqueducts as well as variable hypothyroidism and goiter. In the kidney, pendrin is expressed in the distal nephron and accomplishes HCO_3^- secretion and Cl^- reabsorption. Renal pendrin expression is regulated by acid-base balance. The liver contributes to acid-base regulation by producing or consuming glutamine, which is utilized by the kidney for generation and excretion of NH_4^+, paralleled by HCO_3^- formation. Little is known about the regulation of pendrin in liver. The present study thus examined the expression of Slc26a4 in liver and kidney of mice drinking tap water without or with $NaHCO_3$ (150 mM), NH_4Cl (280 mM) or acetazolamide (3.6 mM) for seven days. As compared to Gapdh transcript levels, Slc26a4 transcript levels were moderately lower in liver than in renal tissue. Slc26a4 transcript levels were not significantly affected by $NaHCO_3$ in liver, but significantly increased by $NaHCO_3$ in kidney. Pendrin protein expression was significantly enhanced in kidney and reduced in liver by $NaHCO_3$. Slc26a4 transcript levels were significantly increased by NH_4Cl and by acetazolamide in liver, and significantly decreased by NH_4Cl and by acetazolamide in kidney. NH_4Cl and acetazolamide reduced pendrin protein expression significantly in kidney, but did not significantly modify pendrin protein expression in liver. The observations point to expression of pendrin in the liver and to opposite effects of acidosis on pendrin transcription in liver and kidney.

Key words: Slc26a4, acidosis, alkalosis, liver, kidney.

Introduction

SLC26A4 encodes pendrin, an anion exchanger transporting chloride, bicarbonate, iodide and a variety of further anions [Choi et al., 2011]. Loss of function defects of SLC26A4 [Dossena et al., 2009] result in autosomal-recessive Pendred syndrome (PDS), characterized by sensorineural hearing loss and enlarged vestibular aqueducts [Choi et al., 2011; Lang et al., 2007; Maciaszczyk and Lewinski 2008] as well as an iodide organification defect with enhanced susceptibility to develop goiter and hypothyroidism [Bizhanova and Kopp 2009; Calebiro et al., 2010; Choi et al., 2011]. The development of goiter and hypothyroidism vary and partially but not fully depends on nutritional iodide intake [Bizhanova and Kopp 2010; Calebiro et al., 2010].

Slc26a4 expression is most abundant in the thyroid gland, the inner ear, and the kidney [Bizhanova and Kopp 2009; Bizhanova and Kopp 2010]. In the thyroid, slc26a4 participates in the transport of iodide [Bizhanova and Kopp 2009; Bizhanova and Kopp 2010]. Slc26a4 is further expressed in lung tissue and participates in the regulation of mucus production [Izuhara et al., 2009]. It has been suggested that IL-13 induced slc26a4 expression contributes to the pathophysiology of bronchial asthma [Izuhara et al., 2009].

In the kidney, slc26a4 is expressed in the apical membrane of type B and non-A, non-B intercalated cells of distal convoluted tubule, connecting tubule and cortical collecting duct and thus accomplishes HCO_3^- secretion and Cl^- reabsorption in the distal nephron [Carraro-Lacroix and Malnic 2010; Sindic and Schlatter 2007; Wagner et al., 2009; Wall and Pech 2010]. Slc26a4 expression and function is increased at enhanced aldosterone or angiotensin II levels and slc26a4 function contributes to the hypertensive effect of those hormones [Eladari et al., 2009; Wall and Pech 2010]. Slc26a4 modifies the expression of the epithelial Na^+ channel ENaC, an effect attributed, at least in part, to its impact on luminal HCO_3^- concentration [Wall and Pech 2010].

Slc26a4 activity and expression in the kidney is regulated by the acid-base status [Hafner et al., 2008; Wagner et al., 2002; Wagner et al., 2009]. Generation of HCO_3^- in type B intercalated cells is accomplished by a cellular carbonic anhydrase (CAII) and a H^+-ATPase at the basolateral membrane [Carraro-Lacroix and Malnic 2010; Sun et al., 2008]. Carbonic anhydrase II deficiency decreases Slc26a4 expression in the kidney [Sun et al., 2008].

Regulation of systemic acid-base balance requires coordinated functions of the liver and the kidneys [Guder et al., 1987]. Acid-base regulation involves renal ammoniagenesis and hepatic urea production, which are tied together by interorgan glutamine flux. Glutamine could be either used in the liver for urea formation or in the kidney for generation and excretion of ammonia [Guder et al., 1987]. Renal ammonia excretion but not hepatic urea formation is paralleled by generation of HCO_3^-. Accordingly, metabolic acidosis is followed by a shift from the use of glutamine for hepatic urea formation to the use of glutamine for renal ammonium formation and excretion [Guder et al., 1987; Karim et al., 2002; Nissim 1999].

Little is known about expression of Slc26a4 in the liver. The present study thus explored whether liver tissue expresses Slc26a4 and whether hepatic Slc26a4 expression is influenced by acid-base balance in a similar way as in the kidney. To this end, Slc26a4 transcript and protein levels were quantified in liver and kidney from mice treated with bicarbonate to induce metabolic alkalosis [Huber et al., 1999], ammonium chloride to trigger metabolic acidosis [Nowik et al., 2010] and acetazolamide to inhibit carbonic anhydrase [Lonnerholm et al., 1986].

Materials and Methods

Animals

Experiments were performed in 8-10 week old female and male C57Bl/6J mice. All animal experiments were conducted according to German and Swiss laws for the welfare of animals and were approved by local authorities. The animals had free access to food (C1310, Altromin, Heidenau, Germany) and tap water. Where indicated $NaHCO_3$ (150 mM), NH_4Cl (280 mM) or acetazolamide (3.6 mM) were added to the drinking water for 7 days. Drinking volumes and body weight were determined prior to and during the experimental period.

Determination of blood pH and pCO_2

Blood samples were collected from the retrobulbar venous plexus to determine pH and pCO_2. The pH was determined utilizing an electrode and pCO_2 utilizing a blood gas analyzer (ABL80 FLEX CO-OX, Radiometer, Willich, Germany). Plasma HCO_3^- concentration was calculated from pCO_2 and pH utilizing the Henderson Hasselbach equation [Harrison 1995].

RT-PCR analysis

To determine Slc26a4 mRNA abundance in mouse liver and kidney RNA was extracted from both tissues using Trifast Reagent (Peqlab, Erlangen, Germany) according to the manufacturer's instructions. Reverse transcription of 2 µg RNA was performed using oligo$(dT)_{12-18}$ primers (Invitrogen, Karlsruhe, Germany) and SuperScript III Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). cDNA samples were treated with RNase H (Invitrogen, Karlsruhe, Germany). Quantitative RT-PCR was performed with the iCycler iQ™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) and iTaq™ Sybr Green Supermix with ROX (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The following primers were used (5'→3' orientation): Slc26a4 s: TTCGGTCTCTACTCTGCCTTT; Slc26a4 as: CCCACCATTAAACTGACCACG; Gapdh s: AGGTCGGTGTGAACGGATTTG; Gapdh as: TGTAGACCATGTAGTTGAGGTCA. The specificity of the PCR products was confirmed by analysis of the melting curves and in addition by agarose gel electrophoresis. All PCRs were performed in duplicate, and mRNA fold changes were calculated by the ΔΔCt method using Gapdh as internal reference.

Membrane preparation and western blot analysis

For total membrane preparation kidney and liver samples were homogenized in an ice-cold K-HEPES buffer (200 mM mannitol, 80 mM HEPES, 41 mM KOH, pH 7.5) containing a protease inhibitor mix (Complete Mini, Roche Diagnostics, Germany) at a final concentration of 1 tablet in a volume of 10 ml solution. Samples were centrifuged at 2000 rpm for 20 min at 4°C. Subsequently, the supernatant was transferred to a new tube and centrifuged at 41000 rpm for 1 h at 4°C. The resultant pellet was resuspended in K-HEPES buffer containing protease inhibitors. After measurement of the total protein concentration (Bio-Rad D_c Protein Assay; Bio-Rad, Hercules, CA, USA), 10 (kidney) or 50 (liver) µg of crude membrane proteins were solubilized in Laemmli sample buffer, and SDS-PAGE was performed on 8% polyacrylamide gels. For immunoblotting, proteins were transferred electrophoretically to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Bedford, MA, USA). After blocking with 5% milk powder in Tris-buffered saline/0.1% Tween-20 for 60 min, the blots were incubated with the respective primary antibodies (rabbit anti-pendrin 1:5000 [Hafner et al., 2008] and mouse monoclonal anti-β-actin antibody (42 kDa; Sigma, St. Louis, MO, USA) 1:5000, diluted in 1%milk/TBS-T) either for 2 h at room temperature or overnight at 4°C. After washing and subsequent blocking, the membranes were incubated for 1 h at room temperature with the secondary antibody: goat anti-rabbit or

anti-mouse IgG-conjugated with alkaline phosphatase 1:5000 (Promega, WI, USA). Antibody binding was detected with the CDP-Star Western chemiluminescence kit (Roche Diagnostics, Mannheim, Germany) using the DIANA III-chemiluminescence detection system (Raytest; Straubenhardt, Germany). All images were analyzed using appropriate software (Advanced Image Data Analyzer, Raytest, Straubenhardt, Germany) to calculate the protein of interest/β-actin ratio.

Statistical analysis

As indicated, data are provided as means ± SEM; *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed t-test, or ANOVA, where applicable. Only results with *($p<0.05$), **($p<0.01$) or ***($p<0.001$) were considered statistically significant.

Results

In order to determine the abundance of mRNA encoding Slc26a4 in hepatic and renal tissue, semi-quantitative reverse transcription polymerase chain reaction (RT-qPCR) was employed. As illustrated in Fig.1A, Slc26a4 mRNA was detected in both, hepatic and renal tissue. mRNA expression was moderately lower in liver than in kidney. The Slc26a4 transcript levels were normalized to the transcript levels of the house keeping gene Gapdh. Again, the Slc26a4/Gapdh transcript level ratio was moderately lower in liver (0.51 ± 0.04, n = 4) than in kidney (1.00 ± 0.04, n = 4). Immunoblotting with antibodies raised against pendrin detected bands in kidney and liver that were not found when the antibody was preincubated with the immunizing peptide (Fig. 1B). Of note, pendrin in liver displayed a slightly higher molecular weight than in liver.

Further experiments were performed to explore the influence of alkalosis and acidosis on Slc26a4 transcript levels in liver and kidney. To this end animals were drinking either plain tap water or either, 150 mM $NaHCO_3$, 280 mM NH_4Cl or 3.6 mM acetazolamide for 7 days. The daily fluid intake approached 4.3 ± 0.1 ml (n = 4), 4.8 ± 0.1 µl (n = 4), 4.1 ± 0.1 µl (n = 4), and 2.1 ± 0.1 µl (n = 4), respectively, in water, $NaHCO_3$, NH_4Cl and acetazolamide drinking animals. The body weight change during the experimental period approached 0.6 ± 0.2 g (n = 4), 0.6 ± 0.2 g (n = 4), 0.4 ± 0.1 g (n =4), and 6.4 ± 0.4 g (n = 4), in water, $NaHCO_3$, NH_4Cl and acetazolamide drinking animals. Plasma pH did not significantly change following drinking of $NaHCO_3$, but significantly decreased following drinking of NH_4Cl or acetazolamide (Table 1).

The switch from water to $NaHCO_3$ did not significantly modify Slc26a4 mRNA expression in liver, but significantly increased Slc26a4 mRNA expression in kidney (Fig. 1C). $NaHCO_3$ significantly increased pendrin protein abundance in kidney and significantly decreased pendrin protein expression in liver (Fig. 2, n = 5/group).

The switch from water to NH_4Cl was followed by a profound increase of Slc26a4 mRNA expression in liver, but to a similarly profound decrease of Slc26a4 mRNA expression in the kidney (Fig. 3). On the protein level, NH_4Cl treatment did not significantly modify pendrin protein expression in liver but significantly reduced pendrin expression in kidney (Fig. 4)

Treatment of the animals with acetazolamide was again followed by a profound increase of Slc26a4 mRNA expression in liver and to a similarly profound decrease of Slc26a4 mRNA expression in the kidney (Fig. 5) Acetazolamide strongly and significantly reduced pendrin protein levels in kidney but did not significantly modify pendrin protein expression in liver (Fig. 6).

Discussion

The present study demonstrates that Slc26a4 transcript and protein levels could be detected in hepatic tissue. The transcript level was moderately lower in liver than in kidney, but the data strongly suggest that substantial amounts of pendrin are expressed in hepatic tissue. Western blotting confirmed the expression of pendrin protein in the liver.

The present observations further demonstrate sensitivity of hepatic and renal Slc26a4 mRNA and protein expression to acidosis and alkalosis. Both, NH_4Cl [Nowik et al., 2010] and acetazolamide [Lonnerholm et al., 1986] are known to cause acidosis, which in turn down-regulates renal Slc26a4 expression [Wagner et al., 2009]. Following acidosis, renal H^+ secretion is up-regulated and renal bicarbonate secretion is down-regulated [Hafner et al., 2008; Wagner et al., 2009]. Since pendrin is inserted into the apical cell membrane and accomplishes HCO_3^- secretion, its operation would be counterproductive for renal acid elimination during acidosis [Carraro-Lacroix and Malnic 2010; Sindic and Schlatter 2007; Wagner et al., 2009; Wall and Pech 2010]. Along those lines, renal Slc26a4 transcript levels are decreased in animals lacking Carbonic anhydrase II [Sun et al., 2008] and its protein abundance is reduced in rats treated with the carbonic anhydrase inhibitor acetazolamide [Welsh-Bacic et al., 2011].

The effect of acidosis on the Slc26a4 transcript levels in liver were opposite to what was observed in the kidney. Both, NH_4Cl and acetazolamide significantly increased pendrin transcript levels in the liver. However, neither NH_4Cl nor acetazolamide significantly modified pendrin protein expression. Thus, it remains uncertain, whether the enhanced pendrin transcript levels indeed translate into the respective alterations of protein expression.

In theory, Slc26a4 may serve a second function in the liver, i.e. cell volume regulation. Parallel activation of Na^+/H^+ exchangers and Cl^-/HCO_3^- exchangers accomplish regulatory cell volume increase [Hoffmann et al., 2009; Lang et al., 1998], as they mediate cellular uptake of NaCl, which in turn osmotically drives water entry. The extrusion of H^+ and HCO_3^- by the Na^+/H^+ exchanger and the Cl^-/HCO_3^- exchanger, respectively, is osmotically not relevant, as they are replenished in the cell from CO_2 via H_2CO_3 [Hoffmann et al., 2009; Lang et al., 1998]. Na^+ accumulated by the Na^+/H^+-exchanger is pumped out by the Na^+/K^+-ATPase in exchange for K^+. Thus, the transporters eventually accomplish cellular KCl-uptake [Hoffmann et al., 2009; Lang et al., 2007].

Cell volume is in turn a decisive regulator of hepatic metabolism. Cell shrinkage stimulates proteolysis and inhibits protein synthesis in the liver, and, conversely, cell swelling stimulates protein synthesis and inhibits proteolysis [Haussinger and Lang 1991; Stoll et al., 1992; vom Dahl et al., 2001; vom Dahl and Haussinger 1995]. Similarly, cell swelling inhibits and cell shrinkage stimulates glycogenolysis [Lang et al., 1989]. As a result, following cell swelling, the intracellular amino acids and glucose are incorporated into and upon cell shrinkage they are released from the osmotically less active macromolecules [Lang et al., 1998]. Cell volume further influences glycolysis, flux through the pentose phosphate pathway, lipogenesis from glucose, gluconeogenesis, oxidation of glycine and alanine, degradation of glutamine, degradation of amino acids to NH_4^+ and urea, ketoisocaproate oxidation, acetyl CoA carboxylase, lipogenesis, carnitine palmitoyltransferase I activity, cytosolic ATP and phosphocreatine concentrations, respiration and RNA and DNA synthesis [Haussinger 2008; Haussinger and Lang 1991; Lang et al., 1998]. Whether or not pendrin indeed participates in cell volume regulatory transport, remains, however, to be tested.

In conclusion, Slc26a4 transcripts and protein were observed in liver tissue. The abundance of both was only moderately lower than in the kidney. The hepatic Slc26a4 transcript levels were upregulated by NH_4Cl and acetazolamide. On protein level, hepatic Slc26a4 decreased in response to NaHCO. Thus, pendrin transcription and/or protein

expression is presumably governed by acid-base balance. The present observations point to a novel role of pendrin, i.e. Cl^-/HCO_3^- exchange in liver cells.

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Table 1: Plasma pH, plasma CO_2 partial pressure (pCO_2, mmHg), as well as plasma Na^+, K^+, and Cl^- concentration (mM) in water, $NaHCO_3$ (150 mM), NH_4Cl (280 mM) and acetazolamide (3.6 mM) drinking animals (arithmetic means ± SEM, n = 5)

Drinking fluid	pH	pCO_2	Na^+	K^+	Cl^-	c
water	7.39 ± 0.01	41.5 ± 0.8	143.2 ± 0.4	4.9 ± 0.2	111.4 ± 1.3	24.9
$NaHCO_3$	7.38 ± 0.01	41.0 ± 2.0	143.2 ± 0.4	4.8 ± 0.1	111.2 ± 0.6	23.9
NH_4Cl	7.29 ± 0.03**	41.2 ± 1.2	147.6 ± 1.2**	4.7 ± 0.1	119.6 ± 0.7***	19.4
Acetazolamide	7.22 ± 0.01***	50.6 ± 1.3***	148.6 ± 0.7***	4.8 ± 0.1	122.0 ± 0.9***	19.9

** ($p<0.01$), *** ($p<0.001$) indicate statistically significant differences to water drinking animals.

Figures

Fig. 1: Slc26a4 mRNA abundance in liver and kidney of water and $NaHCO_3$ drinking animals

A. Representative original bands of mRNA encoding Slc26a4 (upper bands) in kidney (left bands) and liver (right bands). The housekeeping gene Gapdh (lower bands) served as a calibrator and control.

B. Detection of pendrin protein by immunoblotting of mouse kidney and liver. The signal was abolished by preincubation of the immune-serum with the immunizing peptide. The house-keeping protein β-actin served as positive control.

C. Arithmetic means ± SEM of Slc26a4 mRNA abundance in liver (left) (n = 6-8) and kidney (right) (n = 8) of animals drinking water (white bars) or drinking 150 mM $NaHCO_3$ (black bars) for seven days. * ($p<0.05$) indicates statistically significant difference to water drinking animals.

Fig. 2: Slc26a4 protein abundance in liver and kidney from mice exposed to $NaHCO_3$ in drinking water

Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals drinking $NaHCO_3$ in drinking water for 7 days. All blots were stripped and reprobed for β-actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β-actin on the same blot. Arithmetic means ± SEM are shown for n = 5 animals/group. ** ($p<0.01$) and *** ($p<0.001$) indicate statistically significant differences to water drinking animals.

Fig. 3: Slc26a4 mRNA abundance in liver and kidney of water and NH_4Cl drinking animals

Arithmetic means ± SEM of Slc26a4 mRNA abundance in liver (left) (n = 7) and kidney (right) (n = 6-7) of animals drinking water (white bars) or drinking 280 mM NH_4Cl (black bars) for seven days. * ($p<0.05$) indicates statistically significant difference to water drinking animals.

Fig. 4: Slc26a4 protein abundance in liver and kidney from mice receiving NH_4Cl in drinking water

Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals drinking NH_4Cl in drinking water for 7 days. All blots were stripped and reprobed for β-actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β-actin on the same blot. Arithmetic means ± SEM are shown for n = 5 animals/group. *** ($p<0.001$) indicate statistically significant differences to water drinking animals.

Fig. 5: Slc26a4 mRNA abundance in liver and kidney of animals following drinking of water without or with acetazolamide

Arithmetic means ± SEM of Slc26a4 mRNA abundance in liver (left) (n = 7-8) and kidney (right) (n = 7-8) of animals drinking water (white bars) or drinking 3,6 mM acetazolamide (black bars) for seven days. * ($p<0.05$) indicates statistically significant difference to water drinking animals.

Fig. 6: Slc26a4 protein abundance in liver and kidney from mice treated with acetazolamide

Representative original blot for pendrin in liver (left panel) and kidney (right panel) from animals drinking acetazolamide in drinking water for 7 days. All blots were stripped and reprobed for β-actin. Bar graphs summarize data from blots. Pendrin abundance was normalized against β-actin on the same blot. Arithmetic means ± SEM are shown for n = 5 animals/group. *** ($p<0.001$) indicate statistically significant differences to water drinking animals.

Fig. 7: Cartoon illustrating the cooperation of liver and kidney in acidosis (left) and alkalosis (right).

Fig. 1

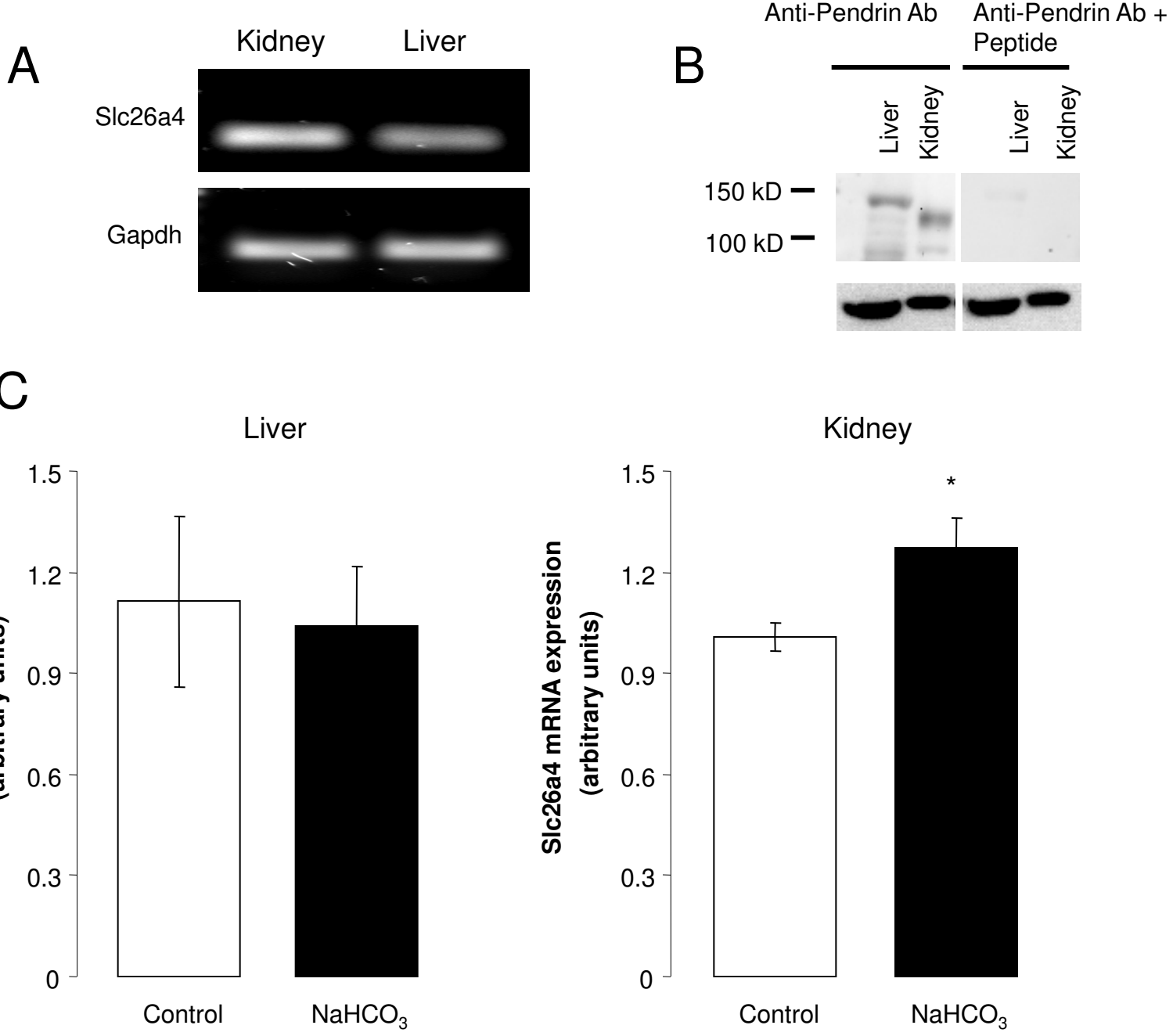

A
Kidney
Liver
Slc26a4
Gapdh
B
Anti-Pendrin Ab
Anti-Pendrin Ab + Peptide
Liver
Kidney
Liver
Kidney
150 kD
100 kD
C
Liver
Kidney
Slc26a4 mRNA expression (arbitrary units)
1.5
1.2
0.9
0.6
0.3
0
*
Control
NaHCO3

Fig. 2

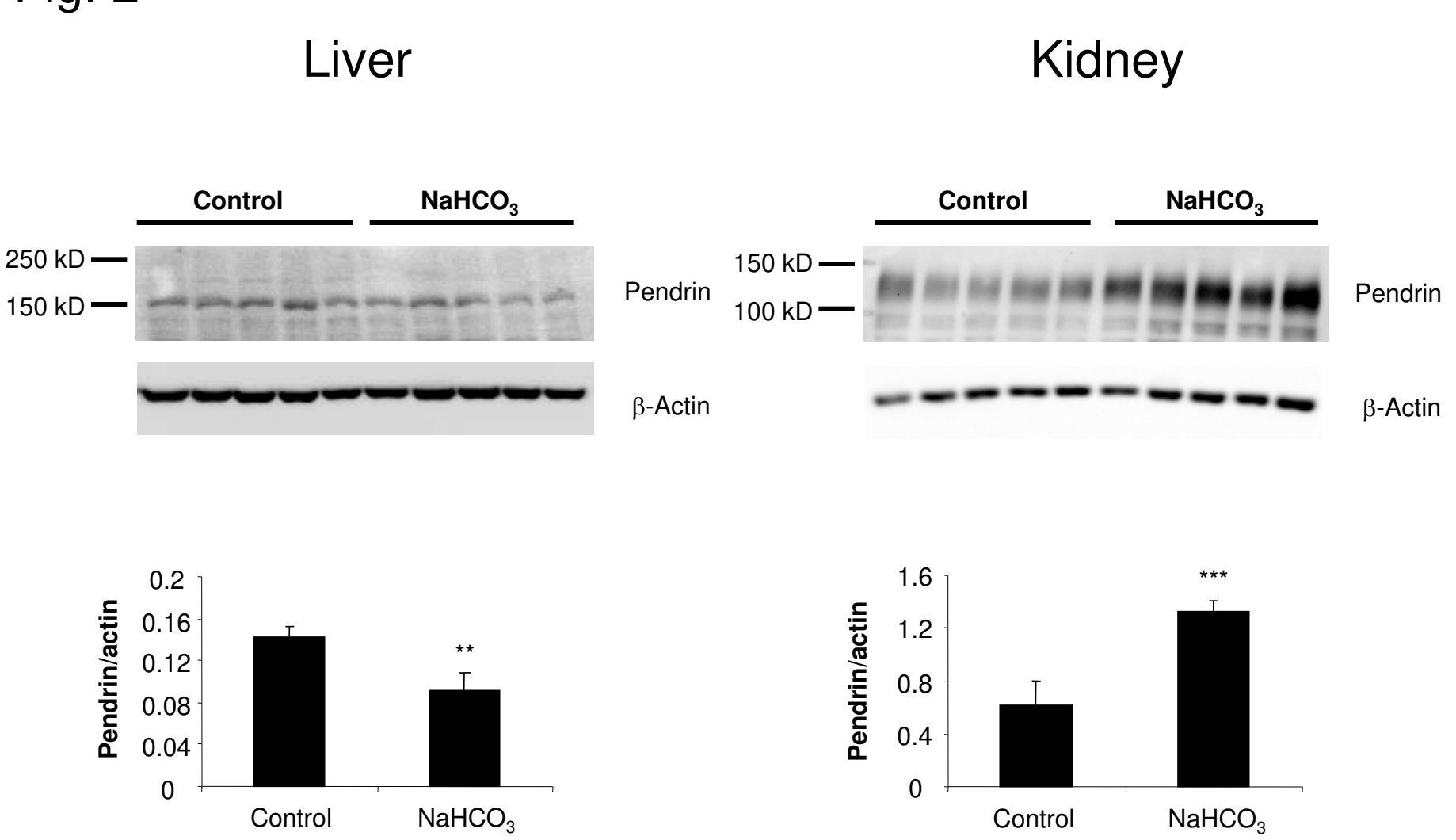

Liver
Kidney
Control
NaHCO3
250 kD
150 kD
Pendrin
β-Actin
150 kD
100 kD
Pendrin/actin
0.2
0.16
0.12
0.08
0.04
0
**
1.6
1.2
0.8
0.4
***
Control
NaHCO3

Fig. 3

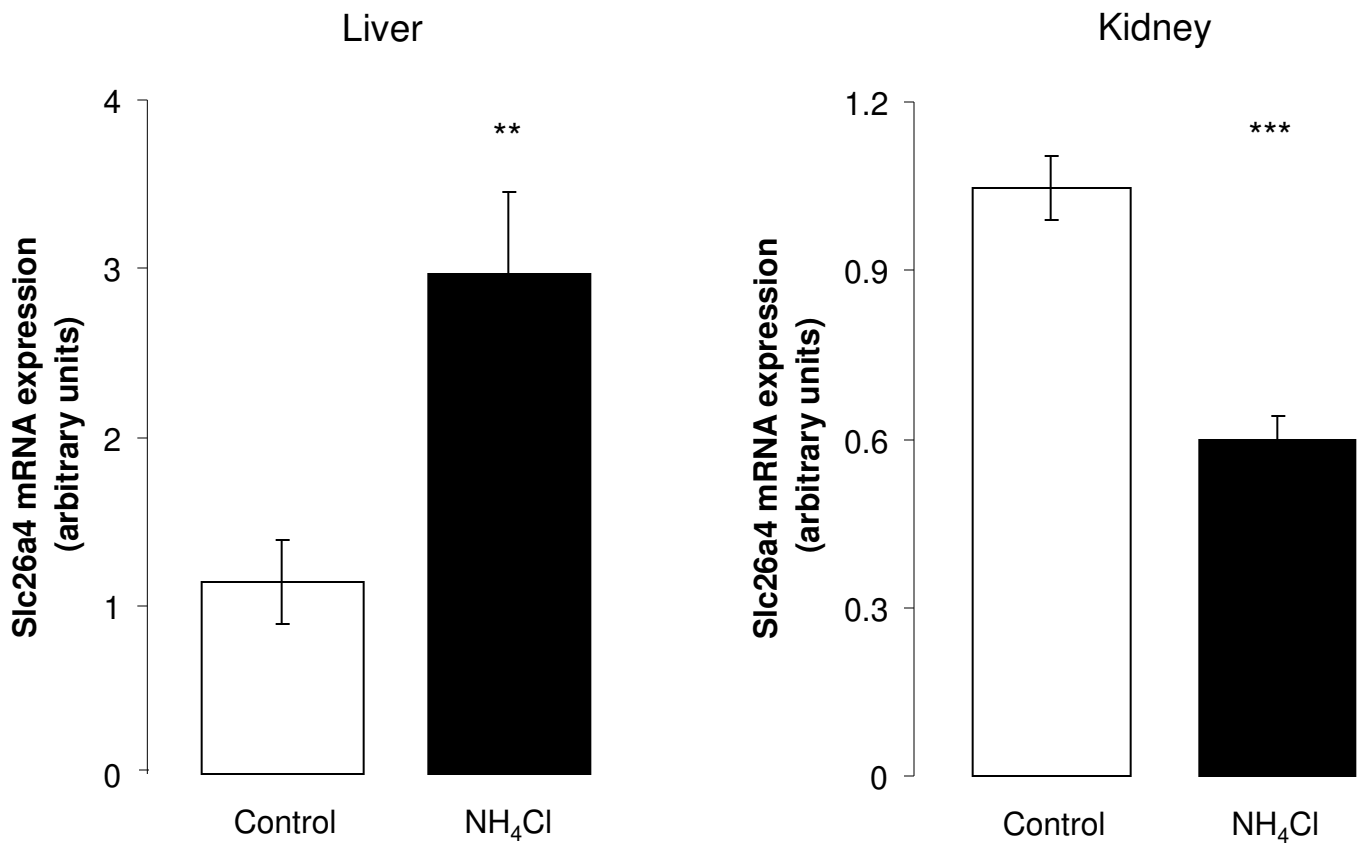

Liver
Slc26a4 mRNA expression (arbitrary units)
4
3
2
1
0
**
Control
NH4Cl
Kidney
Slc26a4 mRNA expression (arbitrary units)
1.2
0.9
0.6
0.3
0
***
Control
NH4Cl

Fig. 4

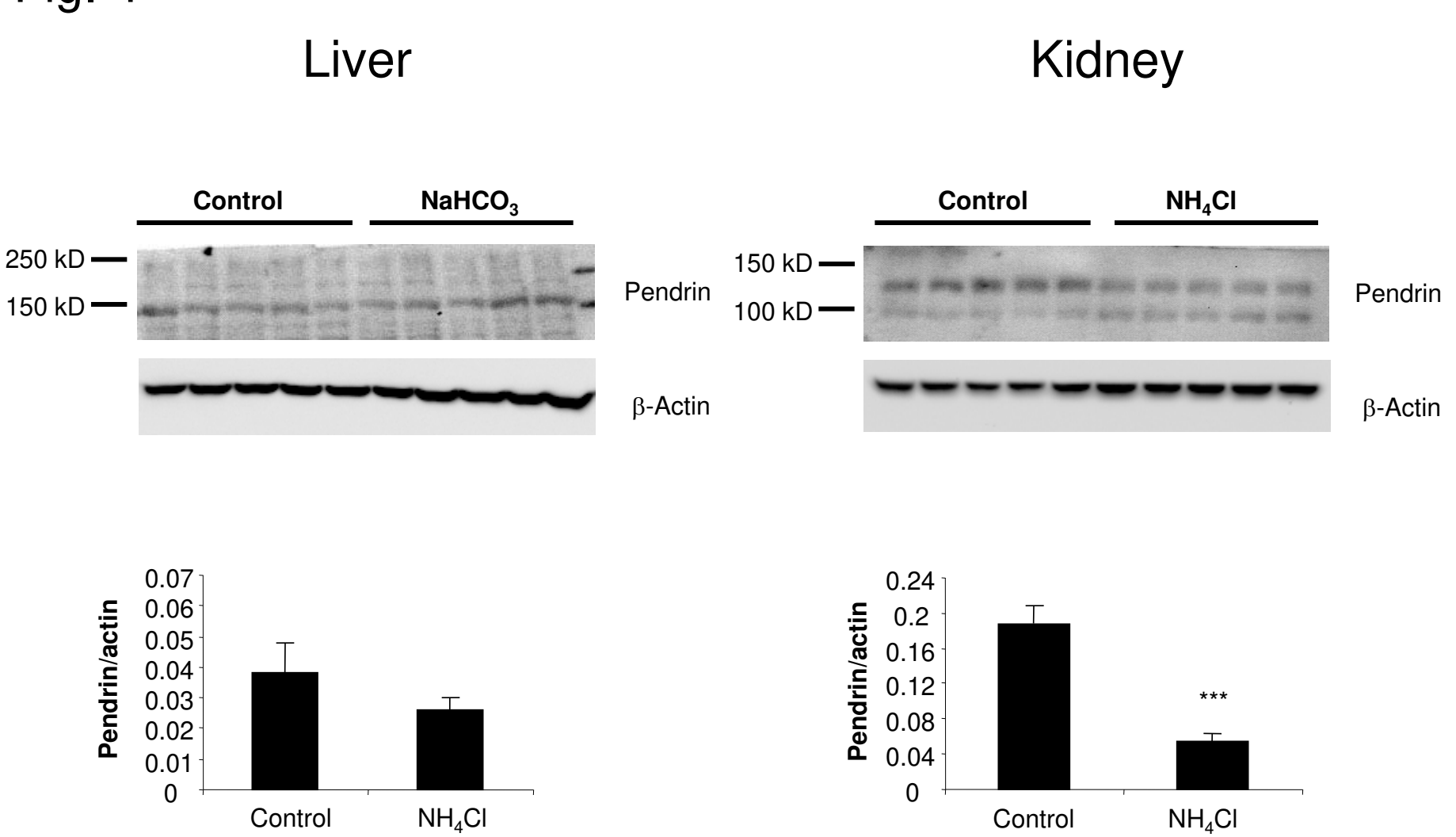

Liver
Kidney
Control
NaHCO3
250 kD
150 kD
Pendrin
β-Actin
Control
NH4Cl
150 kD
100 kD
Pendrin
β-Actin
Pendrin/actin
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Control
NH4Cl
Pendrin/actin
0.24
0.2
0.16
0.12
0.08
0.04
0
***
Control
NH4Cl

Fig. 5

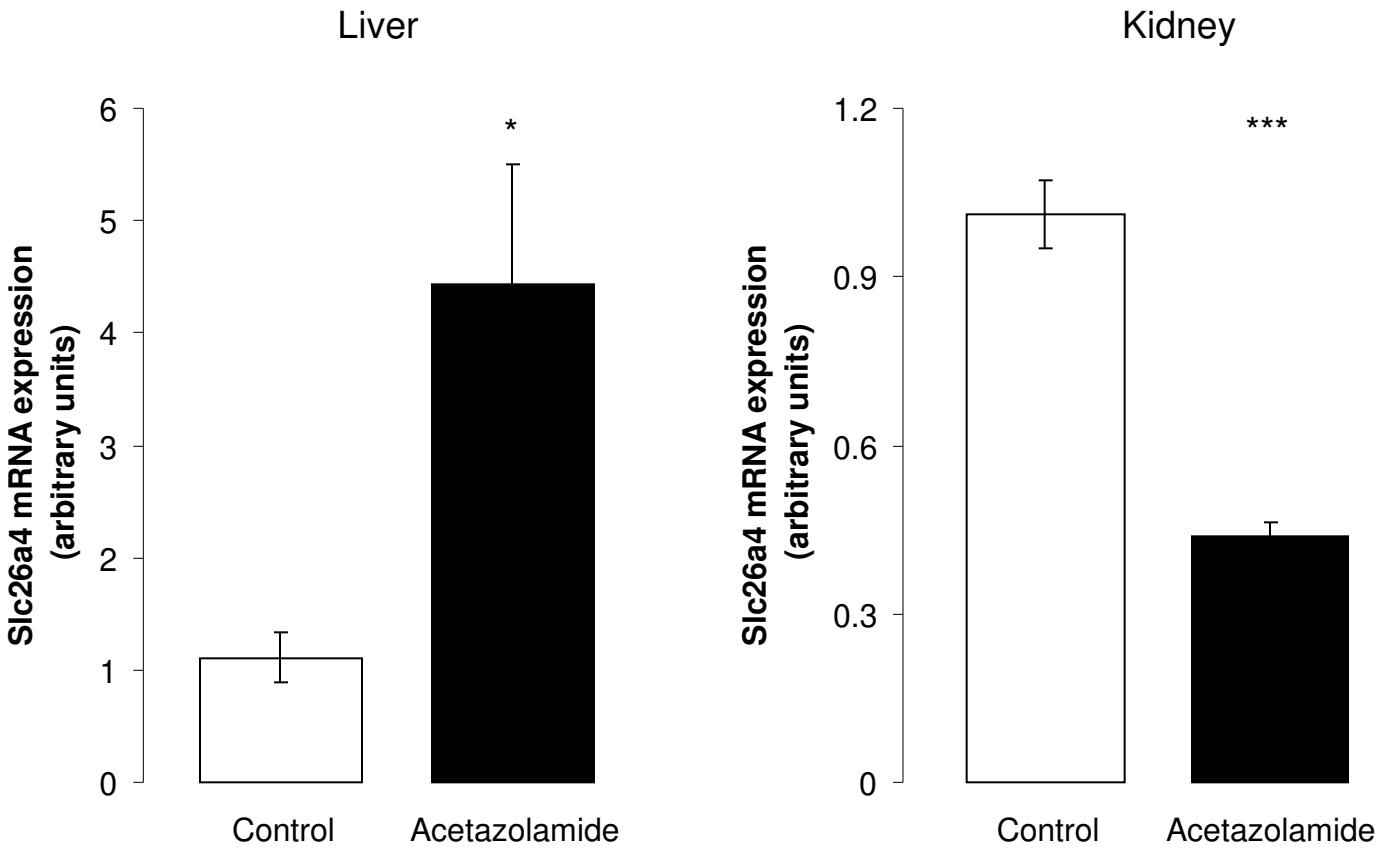
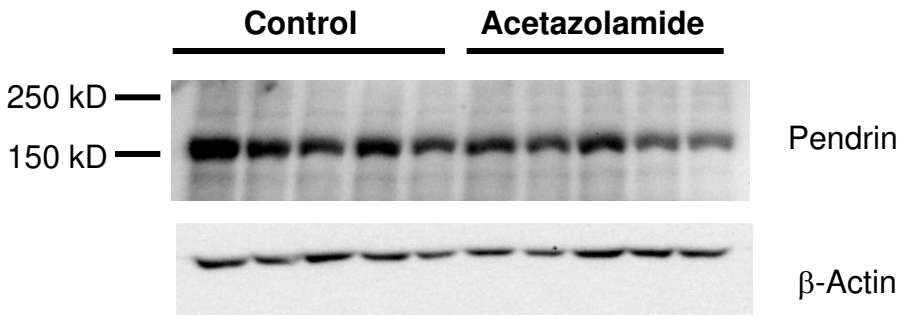
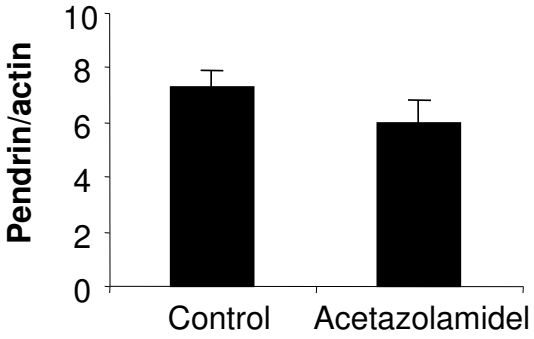

Liver
Slc26a4 mRNA expression (arbitrary units)
6
5
4
3
2
1
0
*
Control
Acetazolamide
Kidney
Slc26a4 mRNA expression (arbitrary units)
1.2
0.9
0.6
0.3
0
***
Control
Acetazolamide

Fig. 6

Liver





Kidney